INTERNATIONAL FILING DATE: 15 MAY 1991

INTERNATIONAL APPLICATION NUMBER: PCT/US 91/03388

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
PCT/US91/03388	15 MAY 1991		ISA/EP		

APPLICANTS: GENETICS INSTITUTE, INC.

UNITED STATES OF AMERICA	525,357	16 MAY 1990
UNITED STATES OF AMERICA	641,204	15 JANUARY 1991

Foreign priority claimed ☐ yes ☐ no
35 USC 119 conditions met ☐ yes ☐ no
Verified and Acknowledged Examiner's Initials

AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
→	025	1	16	8		GI 5132X-P

ADDRESS:
ELLEN J. KAPINOS
LEGAL AFFAIRS
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87 CAMBRIDGEPARK DRIVE
CAMBRIDGE, MASSACHUSETTS 02140

TITLE: BONE AND CARTILAGE INDUCTIVE PROTEINS

CERTIFIED COPY OF PRIORITY DOCUMENT

This is to certify that annexed hereto is a true copy of the above-identified International application as originally filed and any correctious thereto from the records of the United States Patent and Trademark Office acting as a Receiving Office under the Patent Cooperation treaty.

By authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

L. Bland
Certifying Office

Date AUG 24 1995

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CERTIFIED COPY OF PRIORITY DOCUMENT

14 Rec'd PCT/PTO 16 DEC 1991

PATENT COOPERATION TREATY

DEMAND

UNDER ARTICLE 31 OF THE PATENT COOPERATION TREATY:

APTER II

#19 12/31/91

THE UNDERSIGNED REQUESTS THAT THE INTERNATIONAL APPLICATION SPECIFIED BELOW BE THE SUBJECT OF INTERNATIONAL PRELIMINARY EXAMINATION ACCORDING TO THE PATENT COOPERATION TREATY

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or Agent's File Reference (indicated by applicant if desired): GI5182XPCT

International Application No: PCT/US91/03388

International Filing Date: (15.05.91) 15 MAY 91

(Earliest) Priority Date: (16.05.90) 16 MAY 90

Title of Invention: BONE AND CARTILAGE INDUCTIVE PROTEINS

Box No. II APPLICANT(S). Further applicants are indicated on a continuation sheet [X]

Name *and* address, including postal code and country:

GENETICS INSTITUTE, INC.
87 CambridgePark Drive
Cambridge, Massachusetts 02140
United States of America

"Express Mail" mailing label number: RB611382071

Date of Deposit: December 16, 1991

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R.I. 10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 2023

Eileen Zysens

State of nationality: US

State of residence: US

Telephone number (including area code): (617)876-1170

Telegraphic address:

Teleprinter address:

Name *and* address, including postal code and country:

HEWICK, Rodney M.
16 Woodcliffe Road
Lexington, Massachusetts 02173
United States of America

Inventor Only

State of nationality: Great Britain

State of residence: * US

Box No. III AGENT OR COMMON REPRESENTATIVE (IF ANY); ADDRESS FOR NOTIFICATIONS (IN CERTAIN CASES)

The following named agent or common representative

1. [ ] has been appointed earlier and represents the applicant also for international preliminary examination
2. [ ] is hereby appointed and any earlier appointment of an agent is hereby revoked
3. [X] is hereby appointed, in addition to the agent(s) appointed earlier, for the procedure before the International Preliminary Examining Authority

Name *and* address, including postal code and country:

EISEN, Bruce M.; KAPINOS, Ellen J.; DESROSIER, Thomas J.; CSERR, Luann; MCDANIELS, Patricia M. all from:
Legal Affairs
Genetics Institute, Inc.
87 CambridgePark Drive
Cambridge, Massachusetts 02140
United States of America

If the space below is used instead for an address for notifications, check here [ ]

Telephone number (including area code): (617)876-1170

Telegraphic address:

Teleprinter address:

* If the State of residence is not indicated, it will be assumed that it is the same as the State indicated in the address.

Applicant wishes international preliminary examination to start promptly on the basis of the claims

__ XX __ as filed (amendments under Article 19 have not been made and will not be made)

__ __ as amended under Article 19

__ __ as specified on the attached sheet

Box No. V ELECTION OF STATES

The following designated States are hereby elected (please mark the applicable check-boxes)

Regional Patent

__ XX __ EP **European Patent:** AT Austria. BE Belgium. DE Germany (Federal Republic of). DK Denmark. FR France. GB United Kingdom. IT Italy. LU Luxembourg. NL Netherlands. SE Sweden. and any other State which is a Contracting State of the European Patent Convention and of the PCT (including Chapter II thereof). CH and [LI] Switzerland [and Liechtenstein, ES Spain, GR Greece]

__ __ OA **OAPI Patent:** Benin. Burkina Faso. Cameroon. Central African Republic. Chad. Congo. Gabon. Mali. Mauritania. Senegal. Togo.
and any other State which is a Contracting State of the OAPI and of the PCT (including Chapter II thereof)

National Patent

__	AT	Austria	__	KR	Republic of Korea
__	AU	Australia	__	LK	Sri Lanka
__	BB	Barbados	__	LU	Luxembourg
__	BG	Bulgaria	__	MC	Monaco
__	BR	Brazil	__	MG	Madagascar
XX	CA	Canada	__	MW	Malawi
__	DE	Germany (Federal Republic of)	__	NL	Netherlands
__	DK	Denmark	__	NO	Norway
__	FI	Finland	__	RO	Romania
__	GB	United Kingdom	__	SD	Sudan
__	HU	Hungary	__	SE	Sweden
XX	JP	Japan	__	SU	Soviet Union
__	KP	Democratic People's Republic of Korea	__	US	United States of America

Space reserved for electing States which have become party to the PCT (including Chapter II thereof) or bound by Chapter II of the PCT after the issuance of this sheet:

.....

Box No. VI SIGNATURE

Bruce M Eisen

Bruce M. Eisen
Vice President - Chief Patent Counsel

(The following is to be filled in by the International Preliminary Examining Authority)

1. Date of actual receipt of DEMAND: 14 Rec'd PCT/PTO 16 DEC 1991

2. Adjusted date of receipt of DEMAND due to CORRECTIONS under Rule 60.1(b):

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INTERNATIONAL APPLICATION UNDER THE PATENT COOPERATION TREATY

REQUEST

THE UNDERSIGNED REQUESTS THAT THE PRESENT INTERNATIONAL APPLICATION BE PROCESSED ACCORDING TO THE PATENT COOPERATION TREATY

(The following is to be filled in by the receiving Office)

INTERNATIONAL APPLICATION NO. PCT/US 91/03388

INTERNATIONAL FILING DATE: 15 MAY 1991

PCT INTERNATIONAL

(Stamp) Name of receiving Office and "PCT International Application"

Applicant's or Agent's File Reference (indicated by applicant if desired) GI 5182X-PCT

Box No. I TITLE OF INVENTION

BONE AND CARTILAGE INDUCTIVE PROTEINS

Box No. II APPLICANT (WHETHER OR NOT ALSO INVENTOR); DESIGNATED STATES FOR WHICH HE/SHE/IT IS APPLICANT. Use this box for indicating the applicant or, if there are several applicants, one of them. If more than one person (includes, where applicable, a legal entity) is involved, continue in Box No. III.

The person identified in this box is (check one only): ☐ applicant and inventor* ☒ applicant only

Name *and* address:**

GENETICS INSTITUTE, INC.
87 CambridgePark Drive
Cambridge, Massachusetts 02140
United States of America

DELETED DO US

Telephone number: (including area code) 617-876-1170 Telegraphic address: Teleprinter address: 617-876-5851

Country of nationality: US Country of residence:*** US

The person identified in this box is *applicant* for the purposes of (check one only):

☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the "Supplemental Box"

Box No. III FURTHER APPLICANTS, IF ANY; (FURTHER) INVENTORS, IF ANY; DESIGNATED STATES FOR WHICH THEY ARE APPLICANTS (IF APPLICABLE). A separate sub-box has to be filled in in respect of each person (includes, where applicable, a legal entity). If the following two sub-boxes are insufficient, continue in the "Supplemental Box," (giving there for each additional person the same indications as those requested in the following two sub-boxes) or by using a "continuation sheet."

The person identified in this sub-box is (check one only): ☐ applicant and inventor* ☐ applicant only ☒ inventor only*

Name *and* address:**

HEWICK, Rodney M.
16 Woodcliffe Road
Lexington, Massachusetts 02173
United States of America

If the person identified in this sub-box is *applicant (or applicant and inventor)*, indicate also

Country of nationality: GB Country of residence:

and whether that person is *applicant* for the purposes of (check one only):

☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the "Supplemental Box"

The person identified in this sub-box is (check one only): ☐ applicant and inventor* ☐ applicant only ☒ inventor only*

Name *and* address:**

WANG, Jack H.
522 Lowell Street
Lexington, Massachusetts 02173
United States of America

If the person identified in this sub-box is *applicant (or applicant and inventor)*, indicate also:

Country of nationality: US Country of residence:*** US

and whether that person is *applicant* for the purposes of (check one only):

☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the "Supplemental Box"

* If the person indicated as "applicant and inventor" or as "inventor only" is not an *inventor* for the purposes of all the designated States, give the necessary indications in the "Supplemental box."

** Indicate the name of a natural person by giving his/her family name first followed by the given name(s). Indicate the name of a legal entity by its full official designation. In the address, include both the postal code (if any) and the country (name).

*** If residence is not indicated, it will be assumed that the country of residence is the same as the country indicated in the address.

PCT/US 91/03388

Box No. IV AGENT (IF ANY) OR COMMON REPRESENTATIVE (IF ANY); ADDRESS FOR NOTIFICATIONS (IN CERTAIN CASES). A common representative may be appointed only if there are several applicants and if no agent is or has been appointed; the common representative must be one of the applicants.
The following person (includes, where applicable, a legal entity) is hereby/has been appointed as agent or common representative to act on behalf of the applicant(s) before the competent International Authorities:

Name *and* address, including postal code and country:
KAPINOS, Ellen J.; EISEN, Bruce M.; DESROSIER, Thomas; CSERR, Luann; MCDANIELS, Patricia
Legal Affairs
Genetics Institute, Inc.
87 CambridgePark Drive
Cambridge, Massachusetts 02140
United States of America

If the space below is used instead for an address for notifications, mark here ☐

Telephone number: (including area code) 617-876-1170
Telegraphic address:
Teleprinter address: 617-876-5851

Box No. V DESIGNATION OF GROUPS OF STATES OR STATES (1); CHOICE OF CERTAIN KINDS OF PROTECTION OR TREATMENT. The following designations are hereby made (please mark the applicable check-boxes):

Regional Patent

☒ **EP** **European Patent**(2): **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **DE** Germany (Federal Republic of), **FR** France, **GB** United Kingdom, **IT** Italy, **LU** Luxembourg, **NL** Netherlands, **SE** Sweden,
and any other Contracting State of the European Patent Convention which has become party to the PCT after the issuance of this sheet (specify on dotted line):
DK Denmark, ES Spain, GR Greece

☐ **OA** **OAPI Patent:** Benin, Cameroon, Central African Republic, Chad, Congo, Gabon, Mali, Mauritania, Senegal, Togo, and any other member State of OAPI which has become party to the PCT after the issuance of this sheet; if other OAPI title desired, specify on dotted line(3):

National Patent (if other kind of protection or treatment desired, specify on dotted line(3))

☐ **AT** Austria(3)
☐ **AU** Australia(3)
☐ **BB** Barbados
☐ **BG** Bulgaria(3)
☐ **BR** Brazil(3)
☐ **CH and LI** Switzerland and Liechtenstein
☐ **DE** Germany (Federal Republic of)(3)
☐ **DK** Denmark
☐ **FI** Finland
☐ **GB** United Kingdom
☐ **HU** Hungary
☒ **JP** Japan(3)
☐ **KP** Democratic People's Republic of Korea(3)
☐ **KR** Republic of Korea(3)
☐ **LK** Sri Lanka
☐ **LU** Luxembourg(3)
☐ **MC** Monaco(3)
☐ **MG** Madagascar
☐ **MW** Malawi(3)
☐ **NL** Netherlands
☐ **NO** Norway
☐ **RO** Romania
☐ **SD** Sudan
☐ **SE** Sweden
☐ **SU** Soviet Union(3)
☐ **US** United States of America(3)

Space reserved for designating States (for the purposes of a national patent) which have become party to the PCT after the issuance of this sheet:
CA Canada

(1) The applicant's choice of the order of designations may be indicated by marking the check-boxes with sequential arabic numerals (see also the "Notes to Box No. V").
(2) The selection of particular States for a European patent can be made upon entering the national (regional) phase before the European Patent Office (see also the "Notes to Box No. V").
(3) If another kind of protection or a title of addition or, in the United States of America, treatment as a continuation or a continuation-in-part is desired, specify according to the instructions given in the "Notes to Box No. V."

See notes on accompanying sheet

PCT/US 91/03388

Sheet number 3

Box No. VI PRIORITY CLAIM (IF ANY). The priority of the following earlier application(s) is hereby claimed:

Country (country in which it was filed of national application; one of the countries for which it was filed if regional or international application)	Filing Date (day, month, year)	Application No.	Office of Filing (fill in only if the earlier application is an international application or a regional application)
(1) US	16 May 1990 (16.05.90)	07/525,357	
(2) US	15January1991 (15.01.91)	07/641,204	
(3)			

(Letter codes may be used to indicate country and/or Office of filing)

When the earlier application was filed with the Office which, for the purposes of the present international application, is the receiving Office, the applicant may, *against payment of the required fee*, ask the following:

[X] the receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the above-mentioned earlier application/of the earlier applications identified above by the numbers (insert the applicable numbers) 1 and 2

RO/US

Box No. VII EARLIER SEARCH (IF ANY). Fill in where a search (international, international-type or other) by the International Searching Authority has already been requested (or completed) and the said Authority is now requested to base the international search, to the extent possible, on the results of the said earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.

International application number or number and country (or regional Office) of other application:

International/regional/national filing date

Date of request for search:

Number (if available) given to search request:

Box No. VIII SIGNATURE OF APPLICANT(S) OR AGENT

Bruce M. Eisen

Bruce M. Eisen, Reg. No. 22,847
for Genetics Institute, Inc.

If the present Request form is signed on behalf of any applicant by an agent, a separate power of attorney appointing the agent and signed by the applicant is required. If in such case it is desired to make use of a general power of attorney (deposited with the receiving Office), a copy thereof must be attached to this form.

Box No.IX CHECK LIST (To be filled in by the Applicant)

This international application contains the following number of sheets:

1. request	3	sheets
2. description	45 ~~36~~	sheets
3. claims	4	sheets
4. abstract	1	sheets
5. drawings	1	sheets
Total	54 ~~45~~	sheets

Figure number .... of the drawings (if any) is suggested to accompany the abstract for publication.

This international application as filed is accompanied by the items checked below:

1. [ ] separate signed power of attorney
2. [ ] copy of general power of attorney
3. [ ] priority document(s) (see Box No. VI)
4. [ ] receipt of the fees paid or revenue stamps
5. [ ] cheque for the payment of fees
6. [X] request to charge deposit account
7. [X] other document (specify) Microorganism Sheet, 8 Pages of Sequences, Fee Calculation Sheet, 5¼" Floppy Disk, Cert. under 37 CFR 1.10, Sequence Declaration

(The following is to be filled in by the receiving Office)

1. Date of actual receipt of the purported international application: 05 Rec'd PCT/PTO 15 MAY 1991
2. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
3. Date of timely receipt of the required corrections under Article 11 of the PCT:
4. Drawings [ ] Received [ ] No Drawings

(The following is to be filled in by the International Bureau)

Date of receipt of the record copy:

THIS SHEET DOES NOT COUNT AS A PAGE OF THE INTERNATIONAL APPLICATION

APPLICANT	DOCKET NUMBER	This column for use by receiving Office
Genetics Institute, Inc.	GI 5182X-PCT	
RO/US RECEIPT DATE	INTERNATIONAL APPLICATION NUMBER PCT/US 91/03988	SUBMISSION DATE

UNITED STATES RECEIVING OFFICE FEE CALCULATION SHEET[1]

FEES SUBMITTED OR AUTHORIZED:

I. TRANSMITTAL FEE[2] ..... 170 T — 170

II. SEARCH FEE[3]. . . **International Search to be conducted by (Check one)**

☐ **ISA/US (US PTO)** ..... S1 —

☒ **ISA/EP (Eur. Pat. Off.)** ..... 1,492 S2 — 1492 eq/US ad AP.

III. INTERNATIONAL FEE[4]

BASIC FEE[5]

Indicate the number of SHEETS contained in the international application ~~45~~.

first 30 sheets ..... 502 b_1 — 559

remaining 24 ~~15~~ sheets (multiply excess over 30 by amount of supplement to Basic Fee) X $ ~~1100~~ 10.00 = 150 b_2 — 264

Add amounts entered in boxes b_1 and b_2 and enter total in box B.

This figure is the amount of the BASIC FEE ..... 652 B — 823

DESIGNATION FEES[6]

Indicate the number of DESIGNATED STATES for which National patents have been sought and multiply by the amount of the designation fee 2 X $ ~~135~~ 10.00 = 20 d_1 — 270

Indicate the number of GROUPS of designated States for which regional patents have been sought and multiply by the amount of the designation fee 1 X $ ~~135~~ 10.00 = 10 d_2 — 135

– Note instructions regarding the application of designation fees below –

Add amounts entered in boxes d_1 and d_2 and enter total in box D.

This figure is the amount of the DESIGNATION FEES ..... 30 D — 405

Add amounts entered in boxes B and D, and enter total in box I.

This figure is the amount of the INTERNATIONAL FEE ..... 682 I — 1228

IV. TOTAL FEES SUBMITTED OR AUTHORIZED:

Add amounts entered in boxes T, S and I, and enter total in the total box. This figure is the total amount of the FEES SUBMITTED or AUTHORIZED ..... 2344.00 TOTAL — 2890

Payment must be made in United States currency. Checks, postal money orders or bank drafts must be made payable to the Commissioner of Patents and Trademarks. Payment may also be made by authorization to charge to a Patent and Trademark Office deposit account.

DEPOSIT ACCOUNT AUTHORIZATION[7]

☒ **The RO/US is hereby authorized to charged the total fees indicated above to my deposit account.**

☒ **The RO/US is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.**

☒ **The RO/US is hereby authorized to charge my deposit account for preparation, certification and transmittal of the priority document(s) identified in Box VI of the Request form.**

07-1060 — *Deposit Account Number*

May 15, 1991 — *Date*

Bruce M. Eisen — *Signature* Bruce M. Eisen, Reg. No. 22,847

INSTRUCTIONS REGARDING DESIGNATION FEES:

Use the space below to indicate, in order, those countries for which the designation fees submitted or authorized are to be applied. Include after the name of the country any indication that a regional patent is sought. If no countries are indicated below, the RO/US will apply the designation fees submitted or authorized to the designated countries in the order in which those countries are listed in the Request.

BONE AND CARTILAGE INDUCTIVE PROTEINS

This application is a continuation-in-part of U.S. Serial No. 07/525,357 filed May 16, 1990 and U.S. Serial No. 07/641,204 filed January 15, 1991.

The present invention relates to a family of purified proteins which may exhibit the ability to induce cartilage and/or bone formation and processes for obtaining them. These proteins may be used to induce bone and/or cartilage formation and in wound healing and tissue repair.

The invention provides a novel family of proteins termed BMP-8 proteins. Bovine and perhaps other species BMP-8 proteins are characterized by comprising at least one of the same or substantially the same amino acid sequences comprising

(1). Arg-His-Glu-Leu-Tyr-Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr (SEQ ID NO: 1)

(2). Leu-Ser-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg (SEQ ID NO: 2)

(3). Ala-Cys-Cys-Ala-Pro-Thr-Lys (SEQ ID NO: 3)

(4). Thr-Asn-Glu-Leu-Pro-Pro-Pro-Asn-Lys-Leu-Pro-Gly-Ile-Phe-Asp-Asp-Val-His-Gly-Ser-His-Gly-Arg (SEQ ID NO: 4)

The BMP-8 proteins of the invention may be further characterized by an apparent molecular weight of 28,000 - 38,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Under reducing conditions in SDS-PAGE the protein reveals a region of

approximately 14,000-20,000 daltons.

The BMP-8 proteins may be further characterized by a DNA sequence encoding BMP-8 comprising at least one of the following DNA sequences

(1).
GTG CAC CTG CTG AAG CCG CAC GCG GTC CCC AAG GCG TGC TGC GCG CCC ACC AAG CTG AGC GCC ACT TCC GTG CTC TAC TAC GAC AGC AGC AAC AAC GTC ATC CTG CGC AAG CAC CGC AAC ATG GTG GTC CGC GCC TGC GGC TGC CAC (SEQ ID NO: 7)

(2)
GAC TGG GTC ATC GCC CCC CAA GGC TAC TCA GCC TAT TAC TGT GAA GGG GAG TGC TCC TTC CCG CTG GAC TCC TGC ATG AAC GCC ACC AAC CAC GCC ATC CTG CAG TCC CTG (SEQ ID NO: 9)

(3)
GAC GTC CAC GGC TCC CAC GGC CGG CAG GTG
TGC CGT CGG CAC GAG CTG AGC TTC CAG GAC CTG GGC TGG CTG (SEQ ID NO: 11)

It is contemplated that the proteins of the invention are capable of stimulating, promoting, or otherwise inducing cartilage and/or bone formation.

The amino acid sequences set forth above are derived from a bovine bone preparation as further described herein. Based on knowledge of other "BMP" proteins it is expected that the human sequence is the same or homologous to the bovine sequences. The invention further includes human BMP-8 protein and the DNA encoding human BMP-8 as disclosed herein in deposit #75010.

The invention further includes methods for obtaining the DNA sequences encoding the BMP-8 proteins of the invention. This method entails utilizing the above amino acid sequences or portions thereof to design probes to screen libraries for

the human gene or fragments thereof using standard techniques.

The proteins of the invention may be produced by culturing a cell transformed with a DNA sequence encoding the BMP-8 protein and recovering and purifying from the culture medium a protein characterized by comprising at least one of the same or substantially the same amino acid sequences comprising

(1). Arg-His-Glu-Leu-Tyr-Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr (SEQ ID NO: 1)

(2). Leu-Ser-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg (SEQ ID NO: 2)

(3). Ala-Cys-Cys-Ala-Pro-Thr-Lys (SEQ ID NO: 3)

(4). Thr-Asn-Glu-Leu-Pro-Pro-Pro-Asn-Lys-Leu-Pro-Gly-Ile-Phe-Asp-Asp-Val-His-Gly-Ser-His-Gly-Arg (SEQ ID NO: 4)

The expressed protein is isolated, recovered and purified from the culture medium. The purified expressed protein is substantially free from other proteinaceous materials with which it is co-produced, as well as from other contaminants. The recovered purified protein is contemplated to exhibit cartilage and/or bone formation activity.

The proteins of the invention may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described below. It is further contemplated that the proteins of the invention demonstrate activity in this rat bone formation assay at a concentration of .5μ - 500μg/gram of bone formed. It is further contemplated that these proteins demonstrate activity in this assay at a concentration of 1μg - 50μg/gram bone. More particularly, it is contemplated these proteins may be characterized by the ability of 1μg of the protein to score at

least +2 in the rat bone formation assay.

Another aspect of the invention provides pharmaceutical compositions containing a therapeutically effective amount of a protein of the invention in a pharmaceutically acceptable vehicle or carrier. The compositions of the invention may be used to induce bone and/ or cartilage formation. These compositions may also be used for wound healing and tissue repair. Further compositions of the invention may include in addition to a protein of the present invention at least one other therapeutically useful agent such as the proteins designated BMP-1, BMP-2 (also previously referred to as BMP-2A or BMP-2 Class I), BMP-3, BMP-4 (also previously referred to as BMP-2B or BMP-2 Class II) disclosed in PCT publications WO 88/00205 and WO 89/10409; and BMP-5, BMP-6, and BMP-7 disclosed in PCT publication WO 90/11366.

Other therapeutically useful agents include growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), and transforming growth factors (TGF-α and TGF-β). The compositions of the invention may also include an appropriate matrix, for instance, for supporting the composition and/or providing a surface for bone and/or cartilage growth. The matrix may provide slow release of the BMP protein and or the appropriate environment for presentation of the BMP protein.

The compositions may be employed in methods for treating a number of bone and/or cartilage defects, and periodontal disease. They may also be employed in methods for treating various types of wounds and in tissue repair. These methods, according to the invention, entail administering to a patient needing such bone and/or cartilage formation, wound healing or tissue repair, a therapeutically effective amount of a protein of the invention. These methods may also entail the administration of a protein (or portion thereof) of the invention in conjunction with at least one of the "BMP" proteins (or portion thereof) disclosed in the co-owned applications described above. In addition, these methods may

also include the administration of a protein of the invention with other growth factors including EGF, FGF, TGF-a, and TGF-b.

Still a further aspect of the invention are DNA sequences coding for expression of a BMP-8 protein of the invention. Such sequences include a sequence of nucleotides encoding at least one of the same or substantially the same peptide sequences reported above or fragments thereof.

A further aspect of the invention provides vectors containing a DNA sequence encoding BMP-8 proteins of the invention as described above in operative association with an expression control sequence therefor. Host cells transformed with such vectors for use in producing BMP-8 proteins are also provided by the present invention. The host cells containing DNA sequences encoding BMP-8 may be employed in a novel process for producing a protein of the invention. The transformed host cells are cultured in a suitable culture medium and a protein of the invention is isolated and purified from the cells, cell lysate, or conditioned medium by conventional techniques. This process may employ a number of known cells, both prokaryotic and eukaryotic, as host cells for expression of the polypeptide.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description and preferred embodiments thereof.

Brief Description of the Drawing

FIG. 1 illustrates an SDS-PAGE analysis of an osteoinductive fraction (28,000-38,000 daltons non-reduced) following reduction with dithiothreitol.

Detailed Description of the Invention

A purified BMP-8 cartilage/bone protein of the present invention is characterized by comprising at least one of the same or substantially the same amino acid sequences comprising

(1). Arg-His-Glu-Leu-Tyr-Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr (SEQ ID NO: 1)

(2). Leu-Ser-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg (SEQ ID NO: 2)

(3). Ala-Cys-Cys-Ala-Pro-Thr-Lys (SEQ ID NO: 3)

(4). Thr-Asn-Glu-Leu-Pro-Pro-Pro-Asn-Lys-Leu-Pro-Gly-Ile-Phe-Asp-Asp-Val-His-Gly-Ser-His-Gly-Arg (SEQ ID NO: 4)

Purified BMP-8 proteins are substantially free from proteinaceous materials with which they are co-produced as well as from other contaminants. These proteins may be further characterized by the ability to induce cartilage and/or bone formation. It is contemplated that this activity may be demonstrated by activity in the rat bone formation assay as described in Example III. It is further contemplated that these proteins demonstrate activity in the assay at a concentration of .5μ - 500μg/gram of bone formed. It is further contemplated that these proteins demonstrate activity in this assay at a concentration of 1μg - 50μg/gram bone. The proteins may be further characterized by the ability of 1μg to score at least +2 in this assay using either the original or modified scoring method.

The proteins of the invention are further characterized by an apparent molecular weight of 28,000 - 38,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel

electrophoresis (SDS-PAGE). Under reducing conditions in SDS-PAGE the protein reveals a region of approximately 14,000-20,000 daltons.

In a further aspect, the invention provides a method for obtaining the DNA sequences encoding BMP-8 bone/cartilage proteins of the invention. The method for obtaining the DNA sequences entails utilizing the amino acid sequences described above to design probes to screen libraries using standard techniques. The bovine sequence or the human gene thus identified may also be used as a probe to identify a human cell line or tissue which synthesizes the analogous cartilage/bone protein. A cDNA library is synthesized and screened with probes derived from the human or bovine coding sequences. The human sequence thus identified is transformed into a host cell, the host cell is cultured and the protein recovered, isolated and purified from the culture medium. The purified protein is predicted to exhibit cartilage and/or bone formation activity in the rat bone formation assay of Example III.

The proteins provided herein also include factors encoded by the above described sequences but into which modifications are naturally provided (e.g. allelic variations in the nucleotide sequence which may result in amino acid changes in the polypeptide) or deliberately engineered. Similarly, synthetic polypeptides which wholly or partially duplicate continuous sequences of the amino acid residues of the proteins of the BMP-8 proteins are encompassed by the invention. These sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with other cartilage/bone proteins of the invention may possess bone and/or cartilage growth factor biological properties in common therewith. Thus, they may be employed as biologically active substitutes for naturally-occurring proteins in therapeutic processes.

Other specific mutations of the sequences of the proteins of the invention described herein involve modifications of the

glycosylation site. These modifications may involve O-linked or N-linked glycosylation sites. For instance, the absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at the asparagine-linked glycosylation recognition sites present in the sequences of the proteins of the invention. The asparagine-linked glycosylation recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Expression of such altered nucleotide sequences procedures variants which are not glycosylated at that site.

The present invention also encompasses the novel DNA sequences, free of association with DNA sequences encoding other proteinaceous materials, and coding on expression for the proteins of the invention. Further included are those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences isolated in accordance with the procedure described above and demonstrate cartilage and/or bone formation activity in the rat bone formation assay. An example of one such stringent hybridization condition is hybridization at 4 x SSC at 65°C, followed by a washing in 0.1 x SCC at 65°C for an hour. Alternatively, an exemplary stringent hybridization condition is in 50% formamide, 4 x SCC at 42°C.

Similarly, DNA sequences isolated as described above which encode BMP-8 proteins, but which differ in codon sequence due to the degeneracies of the genetic code or allelic variations

(naturally-occurring base changes in the species population which may or may not result in an amino acid change) also encode the proteins of the invention described herein. Variations in the DNA sequences which are caused by point mutations or by induced modifications (including insertion, deletion, and substitution) to enhance the activity, half-life or production of the polypeptides encoded thereby are also encompassed in the invention.

Another aspect of the present invention provides a novel method for producing the proteins of the invention. This method involves culturing a suitable cell line, which has been transformed with a DNA sequence coding for expression of a protein of the invention, under the control of known regulatory sequences. Regulatory sequences include promoter fragments, terminator fragments and other suitable sequences which direct the expression of the BMP-8 protein in an appropriate host cell. A purified BMP-8 protein of the present invention is recovered, isolated and purified from the culture medium. The purified protein is characterized by comprising at least one of the same or substantially the same amino acid sequences comprising

(1). Arg-His-Glu-Leu-Tyr-Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr (SEQ ID NO: 1)

(2). Leu-Ser-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg (SEQ ID NO: 2)

(3). Ala-Cys-Cys-Ala-Pro-Thr-Lys (SEQ ID NO: 3)

(4). Thr-Asn-Glu-Leu-Pro-Pro-Pro-Asn-Lys-Leu-Pro-Gly-Ile-Phe-Asp-Asp-Val-His-Gly-Ser-His-Gly-Arg (SEQ ID NO: 4)

Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U.S. Patent 4,419,446. Another suitable mammalian cell line, which is described in the accompanying examples, is the monkey COS-1 cell line. The mammalian cell CV-1 may also be suitable. Further exemplary mammalian host cells include particularly primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to , HeLa, mouse L-929 cells, 3T3 lines derived form Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines.

Bacterial cells may also be suitable hosts. For example, the various strains of E. coli (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Pseudomonas, other bacilli and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art may also be available as host cells for expression of the polypeptides of the present invention. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g. Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein.

Another aspect of the present invention provides vectors for use in the method of expression of the proteins of the invention. Preferably the vectors contain the full novel BMP-

8 DNA sequences described above which code for the novel cartilage/bone proteins of the invention. Additionally, the vectors also contain appropriate expression control sequences permitting expression of the protein sequences. Alternatively, vectors incorporating truncated or otherwise modified sequences as described above are also embodiments of the present invention and useful in the production of the proteins of the invention. The vectors may be employed in the method of transforming cell lines and contain selected regulatory sequences in operative association with the DNA coding sequences of the invention which are capable of directing the replication and expression thereof in selected host cells. Useful regulatory sequences for such vectors are known to those skilled in the art and may be selected depending upon the selected host cells. Such selection is routine and does not form part of the present invention. The components of the vectors, e.g. replicons, selection genes, enhancers, promoters, and the like, may be obtained from natural sources or synthesized by known procedures. See, Kaufman et al, J. Mol. Biol., 159:511-521 (1982); and Kaufman, Proc. Natl. Acad. Sci., USA, 82:689-693 (1985). Host cells transformed with such vectors and progeny thereof for use in producing cartilage/bone proteins are also provided by the invention.

A protein of the present invention, which induces cartilage and/or bone formation in circumstances where bone and/or cartilage is not normally formed, has application in the healing of bone fractures and cartilage defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery. A protein of the invention may be used in the treatment of

periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g. European patent applications 148,155 and 169,016 for discussions thereof.

The proteins of the invention may also be used in wound healing and related tissue repair. The types of wounds include, but are not limited to burns, incisions and ulcers. (See, e.g. PCT Publication WO84/01106 for discussion of wound healing and related tissue repair).

A further aspect of the invention includes therapeutic methods and composition for repairing fractures and other conditions related to bone and/or cartilage defects or periodontal diseases. In addition, the invention comprises therapeutic methods and compositions for wound healing and tissue repair. Such compositions comprise a therapeutically effective amount of at least one of the proteins of the invention in admixture with a pharmaceutically acceptable vehicle, carrier or matrix. It is expected that the proteins of the invention may act in concert with or perhaps synergistically with one another or with other related proteins and growth factors. Therapeutic methods and compositions of the invention therefore comprise one or more of the proteins of the present invention. Further therapeutic methods and compositions of the invention therefore comprise a therapeutic amount of at least one protein of the invention with a therapeutic amount of at least one of the other "BMP" proteins disclosed in co-owned and co-pending U.S. applications described above. Such methods and compositions of the invention may comprise proteins of the invention or portions thereof in combination with the above-mentioned "BMP" proteins or portions thereof. Such combination may comprise individual molecules from each of the proteins or heteromolecules formed

by portions of the respective proteins. A method and composition of the invention may therefore comprise a protein of the invention or a portion thereof linked with a portion of a different "BMP" as described above protein to form a heteromolecule. For example, a BMP-8 subunit may be linked to a subunit of BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7 or other BMP proteins. Such linkage may comprise disulfide bonds.

Further therapeutic methods and compositions of the invention comprise the proteins of the invention or portions thereof in combination with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), and insulin-like growth factor (IGF). Portions of these agents may also be used in compositions of the invention.

The preparation and formulation of such physiologically acceptable protein compositions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art. The therapeutic compositions are also presently valuable for veterinary applications due to the apparent lack of species specificity in cartilage and bone growth factor proteins. Domestic animals and thoroughbred horses in addition to humans are desired patients for such treatment with the proteins of the present invention.

The therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of cartilage and/or bone or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Preferably for bone and/or cartilage formation,

the composition would include a matrix capable of delivering the cartilage/bone proteins of the invention to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being reabsorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions of the invention will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of the proteins of the invention. Factors which may modify the action of the proteins of the invention include the amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the size of a wound, type of damaged tissue, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix

used in the reconstitution and the type or types of bone and/or cartilage proteins present in the composition. The addition of other known growth factors, such as EGF, PDGF, TGF-α, TGF-β, and IGF-I to the final composition, may also effect the dosage.

Progress can be monitored by periodic assessment of cartilage and/or bone growth and/or repair. The progress can be monitored, for example, using x-rays, histomorphometric determinations and tetracycline labeling.

The following examples illustrate practice of the present invention in recovering and characterizing bovine cartilage and/or bone proteins of the invention and employing these proteins to recover the corresponding human protein or proteins and in expressing the proteins via recombinant techniques.

EXAMPLE I

Isolation of Bovine Cartilage/Bone Inductive Protein

Ground bovine bone powder (20-120 mesh, Colla-Tec) is prepared according to the procedures of M. R. Urist et al., Proc. Natl Acad. Sci USA, 70:3511 (1973) with elimination of some extraction steps as identified below. Ten kgs of the ground powder is demineralized in successive changes of 0.6N HCl at 4°C over a 48 hour period with vigorous stirring. The resulting suspension is extracted for 4 hours in 26 liters of 0.5M EDTA. The residue is washed two times with distilled water before its resuspension in 10 liters of 4M guanidine hydrochloride [GuCl], 1mM N-ethylmaleimide, 1mM iodoacetic acid, 1mM phenylmethylsulfonyl fluoride as described in Clin. Orthop. Rel. Res., 171: 213 (1982). After 16 to 20 hours the supernatant is removed and replaced with another 6 liters of GuCl buffer. The residue is extracted for another 8 hours. The final extraction with 6 liters of GuCl is carried out for 16 hours.

The crude GuCl extracts are combined, filtered through a Pellicon apparatus with a 0.45mM Durapore tangential flow

filter packet, concentrated approximately 50 times on a Amicon RA2000 apparatus with a 10,000 molecular weight cut-off membrane, and then dialyzed in 20mM Tris, 0.05M NaCl, 6M urea (pH7.1), the starting buffer for the first column. After extensive dialysis the protein is loaded on a 2 liter DEAE cellulose column and the unbound fractions are collected.

The unbound fractions are concentrated and dialyzed against 50mM NaAc, 50mM NaCl (pH 4.6) in 6M urea. The unbound fractions are applied to a carboxymethyl cellulose column. Protein not bound to the column is removed by extensive washing with starting buffer, and the material containing protein having bone and/or cartilage formation activity as measured by the Rosen-modified Sampath - Reddi assay (described in Example III below) desorbed from the column by 50mM NaAc, 0.25mM NaCl, 6M urea (pH 4.6). The protein from this step elution is concentrated 20- to 40- fold, then dialyzed extensively against 80mM KPO_4, 6M urea (pH6.0). The sample is applied to an hydroxylapatite column (IBF) equilibrated in 80mM KPO_4, 6M urea (pH6.0) and all unbound protein is removed by washing the column with the same buffer. Protein having bone and/or cartilage formation activity is eluted with 100mM KPO_4 (pH7.4) and 6M urea.

The protein is diluted 5 fold with a 0.1875 M NaCl, 6 M urea solution to a final concentration of 20 mM KPO_4, 150 mM NaCl, 6 M urea. This material is applied to a heparin - Sepharose column equilibrated in 20mM KPO_4, 150mM NaCl, 6M urea (pH7.4). After extensive washing of the column with starting buffer, a protein with bone and/or cartilage inductive activity is eluted by 20mM KPO_4, 700mM NaCl, 6M urea (pH7.4). This fraction is concentrated 10 - 20 fold, dialyzed against 50mM NaAc, 6M urea (pH4.6), and applied to a Pharmacia MonoS HR column. The column is developed with a gradient to 1.0M NaCl, 50mM NaAc, 6M urea (pH4.6). All fractions with absorbance at 280 mM are pooled. This Mono S step is now believed to be dispensable and will be eliminated in the future. The material

is applied to a 4.7 x 30 cm Waters PrepPak 500 C4 cartridge in 0.1% TFA and the column developed with a gradient to 95% acetonitrile, 0.1% TFA in 100 minutes at 45ml per minute. Fractions were assayed for cartilage and/or bone formation activity.

Aliquots of the appropriate fractions are iodinated by one of the following methods: P. J. McConahey et al, Int. Arch. Allergy, 29:185-189 (1966); A. E. Bolton et al, Biochem J., 133:529 (1973); and D. F. Bowen-Pope, J. Biol. Chem., 237:5161 (1982). The iodinated proteins present in these fractions are analyzed by SDS gel electrophoresis.

EXAMPLE II

Characterization of Bovine Cartilage/Bone Inductive Factor

A. Molecular Weight

Approximately 2.5mg protein from Example I from active BMP containing fractions in 0.1% TFA and approximately 45% acetonitrile, is dried with a Savant Speed Vac concentrator and solubilized with Laemmli sample buffer, loaded onto a 12.5% polyacrylamide gel and subjected to SDS-PAGE [Laemmli, U.K. Nature, 227:680-685 (1970)] without reducing the sample with dithiothreitol. The molecular weight is determined relative to iodinated Bio-Rad molecular weight standards. Following autoradiography of the unfixed gel the approximate 28,000-38,000 dalton band is excised and the protein electrophoretically eluted from the gel (Hunkapillar et al Meth. Enzymol. 91:227-236 (1983)]. Based on similar purified bone fractions as described in the co-pending "BMP" applications described above wherein bone and/or cartilage activity is found in the approximately 28,000-38,000 region, it is inferred that this band comprises bone and/or cartilage inductive fractions.

B. Subunit Characterization

The subunit composition of the isolated bovine bone

protein is also determined. The eluted protein described above is fully reduced and alkylated in 2% SDS using iodoacetate and standard procedures. The fully reduced and alkylated sample is then further submitted to SDS-PAGE on a 12.5% gel and the resulting approximate 14,000-20,000 dalton region having a doublet/triplet appearance located by autoradiography of the unfixed gel. A silver stain [Merril et al, Science, 211 : 1437 (1981)] version of the sample is shown in FIG. 1 along with molecular weight markers. The 14,000-20,000 dalton region is indicated by the bracket. Thus the approximate 28,000-30,000 dalton protein yields a broad region of 14,000-20,000.

EXAMPLE III

Rosen Modified Sampath-Reddi Assay

A modified version of the rat bone formation assay described in Sampath and Reddi, Proc. Natl. Acad. Sci. U.S.A., 80:6591-6595 (1983) is used to evaluate bone and/or cartilage activity of the proteins of the invention. This modified assay is herein called the Rosen-modified Sampath-Reddi assay. The ethanol precipitation step of the Sampath-Reddi procedure is replaced by dialyzing (if the composition is a solution) or diafiltering (if the composition is a suspension) the fraction to be assayed against water. The solution or suspension is then redissolved in 0.1 % TFA, and the resulting solution added to 20mg of rat matrix. A mock rat matrix sample not treated with the protein serves as a control. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted subcutaneously in the abdominal thoracic area of 21 - 49 day old male Long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi et al., Proc. Natl Acad Sci., 69:1601 (1972)].

The other half of each implant is fixed and processed for histological analysis. Glycolmethacrylate sections (1μm) are stained with Von Kossa and acid fuschin or toluidine blue to

score the amount of induced bone and cartilage formation present in each implant. The terms +1 through +5 represent the area of each histological section of an implant occupied by new bone and/or cartilage cells and newly formed bone and matrix. A score of +5 indicates that greater than 50% of the implant is new bone and/or cartilage produced as a direct result of protein in the implant. A score of +4, +3, +2 and +1 would indicate that greater than 40%, 30%, 20% and 10% respectively of the implant contains new cartilage and/or bone.

It is contemplated that the dose response nature of the cartilage and/or bone inductive protein containing samples of the matrix samples will demonstrate that the amount of bone and/or cartilage formed increases with the amount of cartilage/bone inductive protein in the sample. It is contemplated that the control samples will not result in any bone and/or cartilage formation.

As with other cartilage and/or bone inductive proteins such as the above-mentioned "BMP" proteins, the bone and/or cartilage formed is expected to be physically confined to the space occupied by the matrix. Samples are also analyzed by SDS gel electrophoresis and isoelectric focusing followed by autoradiography. The activity is correlated with the protein bands and pI. To estimate the purity of the protein in a particular fraction an extinction coefficient of 1 OD/mg-cm is used as an estimate for protein and the protein is run on SDS PAGE followed by silver staining or radioiodination and autoradiography.

EXAMPLE IV

Bovine Protein Composition

The gel slice of the approximate 14,000-20,000 dalton region described in Example IIB is excised and the protein electrophoretically eluted from the gel (Hunkapillar, et al., Supra.). This isolated protein sample is then depleted of SDS [Simpson, et al., Eur. J. Biochem. 165:21-29 (1987)] by being

bound to a 30 x 2.1 mm Brownlee RP-18 after dilution with 5 volumes of 90% n-propanol. Protein is recovered by eluting with a step of 40% n-proponal, 0.1% TFA. The fractions containing the eluted protein peak are pooled and brought to near dryness in a savant Speed Vac concentrator. The protein is then re-solubilized with 0.1 M ammonium bicarbonate and digested with 1 μg of TPCK - treated trypsin (Worthington) for 16 hours at 37°C. A second 1 μg dose of trypsin was added and digestion continued for another 4 hours. The resultant digest is then subjected to RPHPLC using a C4 Vydac RPHPLC column and 0.1% TFA-water, 0.1% TFA water-acetonitrile gradient. The resultant peptide peaks were monitored by UV absorbance at 214 and 280 nm and subjected to direct amino terminal amino acid sequence analysis using an Applied Biosystems gas phase sequenator (Model 470A). Three tryptic fragments are isolated by standard procedures having the following amino acid sequence as represented by the amino acid standard three-letter symbols and where the amino acid in parentheses indicates uncertainty in the sequence:

(1). Arg-His-Glu-Leu-Tyr-Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr (SEQ ID NO: 1)

(2). Leu-Ser-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg (SEQ ID NO: 2)

(3). Ala-Cys-Cys-Ala-Pro-Thr-Lys (SEQ ID NO: 3)

(4). Thr-Asn-Glu-Leu-Pro-Pro-Pro-Asn-Lys-Leu-Pro-Gly-Ile-Phe-Asp-Asp-Val-His-Gly-Ser-His-Gly-Arg (SEQ ID NO: 4)

The four amino acid sequences identified above share homology with other BMP proteins BMP-2, BMP-3, and BMP-4 disclosed in PCT published applications WO 88/00205 and WO

89/10409, BMP-5, BMP-6, and BMP-7 disclosed in USSN's 437,409, 490,033, and 438,919 filed November 15, 1989, November 15, 1989 and November 17, 1989, respectively. Specifically, the above amino acid sequence

(1). Arg-His-Glu-Leu-Tyr--Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr

shares homology with BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7 which contain the following human homologous sequence:

BMP-2: Arg-His-Pro-Leu-Tyr-Val-Asp-Phe-Ser-Asp-Val-Gly-Trp-Asn-Asp-Trp-Ile-Val-Ala-Pro-Pro-Gly-Tyr

BMP-3: Arg-Arg-Tyr-Leu-Lys-Val-Asp-Phe-Ala-Asp-Ile-Gly-Trp-Ser-Glu-Trp-Ile-Ile-Ser-Pro-Lys-Ser-Phe

BMP-4: Arg-His-Ser-Leu-Tyr-Val-Asp-Phe-Ser-Asp-Val-Gly-Trp-Asn-Asp-Trp-Ile-Val-Ala-Pro-Pro-Gly-Tyr

BMP-5: Lys-His-Glu-Leu-Tyr-Val-Ser-Phe-Arg-Asp-Leu-Gly-Trp-Gln-Asp-Trp-Ile-Ile-Ala-Pro-Glu-Gly-Tyr

BMP-6: Lys-His-Glu-Leu-Tyr-Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Gln-Asp-Trp-Ile-Ile-Ala-Pro-Lys-Glu-Tyr

BMP-7: Lys-His-Glu-Leu-Tyr-Val-Ser-Phe-Arg-Asp-Leu-Gly-Trp-Gln-Asp-Trp-Ile-Ile-Ala-Pro-Glu-Gly-Try

The second amino acid sequence

(2). Leu-(Ser)-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg

shares homology with the following human sequences of these

BMP molecules:

BMP-2: Leu-Ser-Ala-Ile-Ser-Met-Leu-Tyr-Leu-Asp-Glu-Asn-Glu-Lys-Val-Val-Leu-Lys

BMP-3: Met-Ser-Ser-Leu-Ser-Ile-Leu-Phe-Phe-Asp-Glu-Asn-Lys-Asn-Val-Val-Leu-Lys

BMP-4: Leu-Ser-Ala-Ile-Ser-Met-Leu-Tyr-Leu-Asp-Glu-Tyr-Asp-Lys-Val-Val-Leu-Lys

BMP-5: Leu-Asn-Ala-Ile-Ser-Val-Leu-Tyr-Phe-Asp-Asp-Ser-Ser-Glu-Val-Ile-Leu-Lys

BMP-6: Leu-Asn-Ala-Ile-Ser-Val-Leu-Tyr-Phe-Asp-Asp-Asn-Ser-Asn-Val-Ile-Leu-Lys

BMP-7: Leu-Asn-Ala-Ile-Ser-Val-Leu-Tyr-Phe-Asp-Asp-Ser-Ser-Asn-Val-Ile-Leu-Lys

The third amino acid sequence

(3). Ala-Cys-Cys-Ala-Pro-Thr-Lys

shares homology with the following human sequences of these BMP molecules:

BMP-2: Ala-Cys-Cys-Val-Pro-Thr-Glu
BMP-3: Pro-Cys-Cys-Val-Pro-Glu-Lys
BMP-4: Ala-Cys-Cys-Val-Pro-Thr-Glu
BMP-5: Pro-Cys-Cys-Ala-Pro-Thr-Lys
BMP-6: Pro-Cys-Cys-Ala-Pro-Thr-Lys
BMP-7: Pro-Cys-Cys-Ala-Pro-Thr-Gln

The fourth amino acid sequence shares some homology (i.e. Asn-Glu-Leu-Pro-) with BMP-3, disclosed in PCT published applications WO 88/00205 and WO 89/10409.

It is contemplated that the BMP-8 proteins of the invention will be structurally similar to these BMP proteins BMP-2 through BMP-7. It is contemplated that mature BMP-8 proteins comprise a dimer of disulfide linked polypeptide subunits.

EXAMPLE V

Isolation of DNA

DNA sequences encoding BMP-8 proteins may be isolated using various techniques known to those skilled in the art. As described below, oligonucleotide probes may be designed on the basis of the amino acid sequence of the above-identified tryptic fragments and synthesized on an automatic DNA synthesizer. The probes may consist of pools of oligonucleotides or unique oligonucleotides designed from the tryptic sequences according to the method of R. Lathe, J. Mol. Biol. 183(1):1-12 (1985).

Based on the similarity of the three amino acid sequences described above to BMP-2 through BMP-7 it is contemplated that the BMP-8 proteins of the invention may have a structure in which amino acid sequence (3) and amino acid sequence (2) are located immediately adjacent to each other as follows:

Ala-Cys-Cys-Ala-Pro-Thr-Lys-Leu-(Ser)-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg

The following four oligonucleotides are designed on the basis of the amino acid sequence of the above identified tryptic fragment [BMP-8 amino acid sequence (2) Leu-(Ser)-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg] and synthesized on an automated DNA synthesizer.

#1: GACTCTAGAATNACRTTRTTNGANG

#2: GACTCTAGAATNACRTTRTTNGARC

#3: GACTCTAGAATNACRTTRTTRCTNG

#4: GACTCTAGAATNACRTTRTTRCTRC

The first 9 nucleotides of oligonucleotides #1 through #4 (underlined) contain the recognition sequence for the restriction endonuclease XbaI in order to facilitate manipulation of a specifically amplified DNA sequence encoding the BMP-8 protein and thus are not derived from the amino acid sequence (2) presented above.

The following oligonucleotide is designed on the basis of the amino acid sequence of another above identified tryptic fragment [BMP-8 amino acid sequence (3) Ala-Cys-Cys-Ala-Pro-Thr-Lys] and synthesized on an automated DNA synthesizer.

#5: GCGGATCCGCNTGYTGYGCNCCNAC

The first 8 nucleotides of oligonucleotide #5 (underlined) contain the recognition sequence for the restriction endonuclease BamHI and for reasons described above are not derived from the amino acid sequence (3).

The standard nucleotide symbols in the above identified probes are as follows: A,adenosine; C,cytosine; G,guanine; T,thymine; N, adenosine or cytosine or guanine or thymine; R,adenosine or guanine; Y,cytosine or thymine; and H, adenosine or cytosine or thymine.

Oligonucleotides #4 and #5 identified above are utilized as primers to allow the amplification of a specific nucleotide sequence from bovine genomic DNA. The amplification reaction is performed as follows:

Bovine genomic DNA (source: bovine liver) is denatured at

100° C for 5 minutes and then chilled on ice before adding to a reaction mixture containing 200 μM each deoxynucleotide triphosphates (dATP, dGTP, dCTP and dTTP), 10 mM Tris-HCl pH 8.3, 50mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 1.25 units Taq DNA polymerase, 100 pM oligonucleotide #4 and 100 pM oligonucleotide #5. This reaction mixture is incubated at 94° C for 2 minutes and then subjected to thermal cycling in the following manner. 1 minute at 94° C, 1 minute at 40 ° C, 1 minute at 72° C for three cycles then 1 minute at 94° C, 1 minute at 55 ° C, 1 minute at 72° C for thirty-seven cycles, followed by a 7 minute incubation at 72° C.

The DNA which is specifically amplified by this reaction is ethanol precipitated, digested with the restriction endonucleases XbaI and BamHI and subjected to agarose gel electrophoresis. A region of the gel is excised, the DNA is electroeluted and an 80 base pair product is subcloned into the plasmid vector pGEM3 between the XbaI and BamHI sites of the polylinker. DNA sequence analysis of resulting subclones indicates that the specifically amplified DNA sequence product encodes the amino acid sequences set forth in tryptic fragments (2) and (3).

The DNA sequence (SEQ ID NO: 5) and derived amino acid sequence (SEQ ID NO: 6) of this specifically amplified DNA fragment is as follows:

```
(1)                          (24)
GGATCCGCGTGCTGTGCTCCGAC C AAG CTG AGC GCC ACC TCC GTG CTC TAC
                            Lys Leu Ser Ala Thr Ser Val Leu Tyr

        (58)                          (80)
TAC GAC AGCAGCAACAATGTAATTCTAGA
Tyr Asp
```

Nucleotides 1-24 of this sequence comprise a portion of oligonucleotide #5 and nucleotides 58-80 comprise a portion of the reverse compliment of oligonucleotide #4 utilized to perform the specific amplification reaction. Due to the

function of oligonucleotides #4 and #5 in initiating the amplification reaction, they may not correspond exactly to the actual sequence encoding a BMP-8 protein and are therefore not translated in the above amino acid derivation.

The following oligonucleotide probe is designed on the basis of the bovine DNA sequence set forth above above and synthesized on an automated DNA synthesizer:

#6: AAGCTGAGCGCCACCTCCGTGCTCTACTAC

This oligonucleotide probe is radioactively labeled with ^{32}P and employed to screen a bovine genomic library constructed in the vector λ EMBL3. 400,000 recombinants of the bovine genomic library are plated at a density of 8000 recombinants per plate on 50 plates. Duplicate nitrocellulose replicas of the recombinant bacteriophage plaques are made from these plates and amplified. The oligonucleotide probe #6 is hybridized to the amplified nitrocellulose replicas in SHB (Standard Hybridization Buffer)at 65 degrees C and washed with 1X SSC, 0.1 % SDS at 65 degrees C. Eleven positively hybridizing recombinants are obtained and are plaque purified. Bacteriophage plate stocks are made and bacteriophage DNA is isolated from each of the eleven plaque purified recombinants. The oligonucleotide hybridizing region of one of the recombinants, designated λ9800-10 is localized to a 0.4 kb PstI fragment. This fragment is subcloned into a plasmid vector (pGEM-3) and DNA sequence analysis performed. The partial DNA sequence (SEQ ID NO: 7) and derived amino acid sequence (SEQ ID NO: 8) of this region of clone λ9800-10 are shown in Table 1. The bacteriophage λ9800-10 was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD "ATCC" under the accession #75011 on May 15, 1991. This deposit meets the requirements of the Budapest Treaty of the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and Regulations thereunder.

Table 1

```
                                                    30
TGCCCGCTGCCCCCTCCCGCCCCCGCCAG GTG CAC CTG CTG AAG CCG CAC GCG
                                Val His Leu Leu Lys Pro His Ala

GTC CCC AAG GCG TGC TGC GCG CCC ACC AAG CTG AGC GCC ACT TCC GTG
Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val

CTC TAC TAC GAC AGC AGC AAC AAC GTC ATC CTG CGC AAG CAC CGC AAC
Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn

                                                   176
ATG GTG GTC CGC GCC TGC GGC TGC CAC TGA GGCCCCAACTCCACCGGCAG
MET Val Val Arg Ala Cys Gly Cys His
```

It is noted that nucleotide 95 is a "T" whereas in the amplified DNA fragmnet described above the corresponding nucleotide is a "C". This λ9800-10 clone encodes at least a portion of the bovine BMP-8 protein of the invention. The BMP-8 peptide sequence from this clone is 49 amino acids in length and is encoded by the DNA sequence from nucleotide 30 through nucleotide 176. The amino acid sequence corresponding to tryptic fragments (2) and (3) isolated from bovine bone 28 to 30kD material is underlined in Table 1. An in-frame stop codon (TGA) [nucleotides 177-179] indicates that this clone encodes the carboxy-terminal portion of the bovine BMP-8 protein of the invention. The nucleotides 1-29 are believed to be intron sequences based on the presence of a consensus splice site (a pyrimidine-rich stretch followed by the dinucleotide AG) as well as the lack of homology of the potentially encoded amino acids to other BMP proteins.

The following two oligonucleotides are designed on the basis of the amino sequence of tryptic fragment (1) Arg-His-Glu-Leu-Tyr-Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr ((SEQ ID NO: 1) described above.

#7: TGGGTNATHGCNCCNCA

#8: ATHGCNCCNCARGGNTA

These oligonucleotides hybridize to clone λ9800-10 in SHB at 42 degrees C with washing in 5X SSC, 0.1 % SDS at 42 degrees C. A restriction fragment of clone λ9800-10 containing the hybridizing region of both oligo #7 and #8 set forth above is subcloned into a plasmid vector (pGEM-3) and DNA sequence analysis performed. The partial DNA sequence (SEQ ID NO: 9) and derived amino acid sequence (SEQ ID NO: 10) of this region of clone λ9800-10 are shown in Table 2.

Table 2

```
                                                                    51
GGGGTGGGAG GGCACGTGGA TGGGACTCAC CTTCTCCCAC TACCCCCCAG GAC TGG
                                                                   AspTrp

GTC ATC GCC CCC CAA GGC TAC TCA GCC TAT TAC TGT GAA GGG GAG TGC
Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys

TCC TTC CCG CTG GAC TCC TGC ATG AAC GCC ACC AAC CAC GCC ATC CTG
Ser Phe Pro Leu Asp Ser Cys MET Asn Ala Thr Asn His Ala Ile Leu

         161
CAG TCC CTG GTCAGTACCTC
Gln Ser Leu
```

This region of clone λ9800-10 encodes another portion of the bovine BMP-8 protein of the invention. The BMP-8 peptide sequence from this clone is 37 amino acids in length and is encoded by the DNA sequence from nucleotide 51 through nucleotide 161. A portion of the amino acid sequence corresponding to tryptic fragment (1) (SEQ ID NO: 1) isolated from bovine bone 28 to 30kD material is underlined in Table 2.

The nucleotides 1-50 are believed to be intron sequences based on the presence of a consensus splice site and lack of homology of the derived amino acid sequence to the remainder of the tryptic fragment (1). Similarly, the nucleotide sequences 162-172 are also believed to be intron sequences.

Another PstI restriction fragment of clone λ9800-10 is subcloned and sequenced in a similar manner to that described above. The partial DNA sequence (SEQ ID NO: 11) and derived amino acid sequence (SEQ ID NO:12) of this region of clone λ9800-10 are shown in Table 3.

Table 3

```
                    20
CCCTTGCGTGTCCCCGCAG AC GAC GTC CAC GGC TCC CAC GGC CGG CAG GTG
                       Asp Val His Gly Ser His Gly Arg Gln Val

    99
TGC CGT CGG CAC GAG CTG TAC GTG AGC TTC CAG GAC CTG GGC TGG CTG
Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu

GTGAGTTCCGACTCTCCTTT
```

This region of clone λ9800-10 encodes another portion of the BMP-8 protein of the invention. The BMP-8 peptide sequence from this clone is 26 amino acids in length and is encoded by the DNA sequence from nucleotide 20 through nucleotide 99. The remaining portion of the amino acid sequence corresponding to tryptic fragments (1) isolated from bovine bone 28 to 30kD material is underlined in Table 3. It is also noted that this sequence encodes a peptide sequence comprising portion of the tryptic fragment (4) isolated from bovine bone 28 to 30kD material [(Thr)-Asn-Glu-Leu-Pro-Pro-(Pro)-Asn-Lys-Leu-(Pro)-Gly-Ile-Phe-Asp-Asp-Val-His-Gly-Ser-His-Gly-Arg](SEQ ID NO: 4). The amino acid sequence corresponding to this tryptic peptide is also underlined in Table 3. The nucleotide sequences 1-19 and 100-120 are believed to be intron sequences

on the basis of reasons described previously.

Based on the derived amino acid sequences set forth in Tables 1, 2, and 3, the bovine BMP-8 protein of the invention is contemplated to be comprised of the amino acid sequence (SEQ ID NO: 13) present in Table 4.

Table 4

1 11
Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His Glu Leu
Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro
Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu
Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val
His Leu Leu Lys Pro His Ala Val Pro Lys Ala Cys Cys Ala Pro Thr
Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val
Ile Leu Arg Lys His Arg Asn Met Val Val Arg Ala Cys Gly Cys His
112

This sequence is realized to be homologous to other BMP proteins. For example, the carboxy-terminal cysteine-rich region (amino acids #11 through #112 of Table 4) demonstrate the following amino acid identities: 55% to BMP-2; 41% to BMP-3; 55% to BMP-4; 74% to BMP-5; 75% to BMP-6; and 75% to BMP-7.

EXAMPLE V

Human BMP-8

A 0.4 kb PstI bovine genomic BMP-8 fragment comprising the sequence set forth in Table 1 is radioactively labeled with ^{32}P and used as a probe to screen a human genomic library [Strategene Cloning Systems (catalog # 944201)] constructed in the vector λFIX. 1,000,000 recombinants of this human genomic library are plated at a density of 20,000 bacteriophage per plate. Duplicate nitrocellulose replicas of the recombinant bacteriophage plaques are made and hybridized to the bovine

genomic probe in SHB at 65 degrees C and washed with 0.2X SSC, 0.1 % SDS at 65 degrees C. Twenty-five positives are obtained and replated for secondaries.

The following oligonucleotide probe is designed on the basis of nucleotide 57 through nucleotide 86 of the DNA sequence set forth in Table 2 and synthesized on an automated DNA synthesizer.

#9: GTCATCGCCCCCCAAGGCTACTCAGCCTAT

The following oligonucleotide probe is designed on the basis of nucleotide 20 through nucleotide 43 of the DNA sequence set forth in Table 3 and synthesized on an automated DNA synthesizer.

#10: ACGACGTCCACGGCTCCCACGGCC

One set of secondary filters is hybridized to probe #9 in SHB at 65 degrees C and washed in 1X SSC, 0.1% SDS at 65 degrees C, the other set of secondary filters are hybridized to probe #10 in SHB at 50 degrees C and washed in 5X SSC, 0.1% SDS at 50 degrees C. Two clones are found to hybridize to both oligonucleotide probes. The positive hybridization of oligonucleotides #9 and #10 to these two human genomic clones indicates that they contain at least a portion of the nucleotide sequence encoding the human equivalent of the BMP-8 protein of the invention. One of these clones is designated λH8 12-1 and the bacteriophage was deposited with "ATCC" under the accession #75010 on May 15, 1991. This deposit meets the requirements of the Budapest Treaty of the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and Regulations thereunder.

Once a recombinant bacteriophage containing DNA encoding a portion of the human cartilage and/or bone inductive factor molecule is obtained, the human coding sequence can be used as

a probe to identify a human cell line or tissue which synthesizes the bone inductive factor. Alternatively, the bovine coding sequence can be used as a probe to identify such human cell line or tissue. Briefly described, RNA is extracted from a selected cell or tissue source and either electrophoresed on a formaldehyde agarose gel and transferred to nitrocellulose, or reacted with formaldehyde and spotted on nitrocellulose directly. The nitrocellulose is then hybridized to a probe derived from a coding sequence of the bovine or human cartilage and/or bone inductive protein. mRNA is selected by oligo (dT) cellulose chromatography and cDNA is synthesized and cloned in lambda gt10 by established techniques (Toole et al., supra).

Additional methods known to those skilled in the art may be used to isolate the human and other species' cartilage/bone proteins of the invention. The procedures described above may be employed to isolate other related proteins of interest by utilizing the bovine or human proteins as a probe source. Such other proteins may find similar utility in, inter alia, fracture repair, wound healing and tissue repair.

EXAMPLE VI

Expression of the Cartilage/Bone Proteins

In order to produce bovine, human or other mammalian proteins of the invention, the DNA encoding it, isolated as described above, is transferred into an appropriate expression vector and introduced into mammalian cells or other preferred eukaryotic or prokaryotic hosts by conventional genetic engineering techniques. Methods of transfection include electroporation, $CaPO_4$ precipitation, protoplast fusion, microinjection and lipofection. Once the host cells are transformed, stable transformants are then screened for expression of the product by standard immunological, biological or enzymatic assays. The presence of this DNA and mRNA encoding the BMP-8 polypeptides may be detected by standard

procedures such as Southern and Northern blotting, high expressing cell lines are cloned or recloned at the appropriate level of selectivity to obtain a more homologous population of cells.

Selected transformed host cells are cultured and the BMP-8 proteins of the invention expressed thereby are recovered, isolated and purified. Characterization of the expressed proteins is carried out using standard techniques. For example characterization may include pulse labeling with [35 S] methionine or cysteine and analysis by polyacrylamide electrophoresis. The recombinantly expressed BMP-8 proteins are free of proteinaceous materials with which they are coproduced and with which they ordinarily are associated in nature, as well as from other contaminants, such as materials found in the cellular media.

It is contemplated that the preferred expression system for biologically active recombinant human proteins of the invention will be stably transformed mammalian cells. For transient expression the cell line of choices is expected to be SV40 transformed African green monkey kidney COS-1 in COS-7 which typically produce moderate amounts of the protein encoded within the plasmid for a period of 1-4 days. It is further contemplated that the preferred mammalian cells will be CHO cells.

One skilled in the art can construct mammalian expression vectors by employing the DNA sequences of the invention sequences and known vectors, such as pCD [Okayama et al., Mol. Cell Biol., 2:161-170 (1982)] and pJL3, pJL4 [Gough et al., EMBO J., 4:645-653 (1985)]. The transformation of these vectors into appropriate host cells may result in expression of the proteins of the invention. One skilled in the art could manipulate the sequences of the invention by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with bacterial sequences to create bacterial vectors for intracellular or extracellular expression by

bacterial cells. For example, the coding sequences could be further manipulated (e.g. ligated to other known linkers or modified by deleting non-coding sequences there-from or altering nucleotides therein by other known techniques). The modified coding sequence could then be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al., Proc. Natl Acad. Sci. USA, 77:5230-5233 (1980). This exemplary bacterial vector could then be transformed into bacterial host cells and a protein of the invention expressed thereby. For a strategy for producing extracellular expression of a cartilage and/or bone protein of the invention in bacterial cells., see, e.g. European patent application EPA 177,343.

Similar manipulations can be performed for the construction of an insect vector [See, e.g. procedures described in published European patent application 155,476] for expression in insect cells. A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of the factors of the present invention by yeast cells. [See, e.g., procedures described in published PCT application WO86/00639 and European patent application EPA 123,289].

A method for producing high levels of a protein of the invention from mammalian cells involves the construction of cells containing multiple copies of the heterologous gene encoding proteins of the invention. The heterologous gene may be linked to an amplifiable marker, e.g. the dihydrofolate reductase (DHFR) gene for which cells containing increased gene copies can be selected for propagation in increasing concentrations of methotrexate (MTX) according to the procedures of Kaufman and Sharp, J. Mol. Biol., 159:601-629 (1982). This approach can be employed with a number of different cell types. For example, a plasmid containing a DNA sequence for a protein of the invention in operative association with other plasmid sequences enabling expression

thereof and the DHFR expression plasmid pAdA26SV(A)3 [Kaufman and Sharp, Mol. Cell. Biol., 2:1304 (1982)] may be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by calcium phosphate coprecipitation and transfection, electroperation or protoplast fusion. DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum, and subsequently selected for amplification by growth in increasing concentrations of MTX (sequential steps in 0.02, 0.2, 1.0 and 5uM MTX) as described in Kaufman et al., Mol Cell Biol., 5:1750 (1983). Transformants are cloned, and the proteins of the invention are recovered, isolated, and purified from the culture medium. Biologically active protein expression is monitored by the Rosen-modified Sampath - Reddi rat bone formation assay described above in Example III. Protein expression should increase with increasing levels of MTX resistance. Similar procedures can be followed to produce other related proteins.

EXAMPLE VII

Biological Activity of Expressed Cartilage/Bone Proteins

To measure the biological activity of the expressed BMP-8 proteins obtained in Example VI above, the protein may be partially purified on a Heparin Sepharose column and further purified using standard purification techniques known to those skilled in the art. For example, post transfection conditioned medium supernatant collected from the cultures may be concentrated by ultrafiltration, dialyzed and applied to a Heparin Sepharose column.

Further purification may be achieved by preparative $NaDodSO_4$/PAGE [Laemmli, Nature 227:680-685 (1970)]. For instance, the protein is applied to a gel. Recovery may be estimated by adding L-[^{35}S]methionine-labeled BMP protein purified over heparin-Sepharose as described above. Protein may be visualized by copper staining of an adjacent lane [Lee, et al., Anal. Biochem. 166:308-312 (1987)]. Appropriate bands

are excised and extracted.

The appropriate amount of the resulting solution is mixed with 20 mg of rat matrix and then assayed for in vivo bone and/or cartilage formation activity by the Rosen-modified Sampath - Reddi assay. A mock transfection supernatant fractionation is used as a control. The implants containing rat matrix to which specific amounts of human proteins of the invention have been added are removed from rats after seven days and processed for histological evaluation. Representative sections from each implant are stained for the presence of new bone mineral with von Kossa and acid fuschin, and for the presence of cartilage-specific matrix formation using toluidine blue. The types of cells present within the section, as well as the extent to which these cells display phenotype are evaluated and scored as described in Example III.

The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are believed to be encompassed within the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Wang, Jack H.

(ii) TITLE OF INVENTION: Bone and Cartilage Inductive Proteins

(iii) NUMBER OF SEQUENCES: 13

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
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(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 32,245
(C) REFERENCE/DOCKET NUMBER: GI5182X-PCT

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: Bone

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

b)

GAC TGG GTC ATC GCC CCC CAA GGC TAC TCA GCC TAT TAC TGT GAA GGG GAG TGC TCC TTC CCG CTG GAC TCC TGC ATG AAC GCC ACC AAC CAC GCC ATC CTG CAG TCC CTG (SEQ ID NO: 9); and

c)

GAC GTC CAC GGC TCC CAC GGC CGG CAG GTG TGC CGTCGG CAC GAG CTG AGC TTC CAG GAC CTG GGC TGG CTG (SEQ ID NO: 11).

4. A purified protein characterized by the amino acid sequence encoded by the DNA of ATCC #75010.

5. The DNA sequence of ATCC #75010 encoding BMP-8.

6. A purified protein produced by the steps of:

(a) culturing a cell transformed with a vector having a DNA sequence of claim 3 said DNA sequence in operative association with an expression control sequence therefor; and

(b) recovering, isolating and purifying from said culture medium a protein characterized by the ability to induce cartilage and/or bone formation.

7. A purified protein produced by the steps of:

(a) culturing a cell transformed with a vector containing the DNA sequence of ATCC #75010 encoding BMP-8 said sequence

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bos taurus
(F) TISSUE TYPE: Bone

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Thr Asn Glu Leu Pro Pro Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp
1 5 10 15

Val His Gly Ser His Gly Arg
20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 80 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bos taurus

(vii) IMMEDIATE SOURCE:
(B) CLONE: acc30

(viii) POSITION IN GENOME:
(C) UNITS: bp

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 25..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGATCCGCGT GCTGTGCTCC GACC AAG CTG AGC GCC ACC TCC GTG CTC TAC 51
Lys Leu Ser Ala Thr Ser Val Leu Tyr
1 5

TAC GAC AGCAGCAACA ATGTAATTCT AGA 80

Tyr Asp
10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 199 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bos taurus

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Bovine genomic
(B) CLONE: Lambda 9800-10

(viii) POSITION IN GENOME:
(C) UNITS: bp

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 30..199

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 1..29

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 30..179

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGCCCGCTGC CCCCTCCCGC CCCCGCCAG GTG CAC CTG CTG AAG CCG CAC GCG 53
Val His Leu Leu Lys Pro His Ala
1 5

GTC CCC AAG GCG TGC TGC GCG CCC ACC AAG CTG AGC GCC ACT TCC GTG 101

```
Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val
 10                  15                  20

CTC TAC TAC GAC AGC AGC AAC AAC GTC ATC CTG CGC AAG CAC CGC AAC      149
Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn
 25                  30                  35                  40

ATG GTG GTC CGC GCC TGC GGC TGC CAC TGAGGCCCCA ACTCCACCGG            196
Met Val Val Arg Ala Cys Gly Cys His
                45                  50

CAG                                                                  199
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Val His Leu Leu Lys Pro His Ala Val Pro Lys Ala Cys Cys Ala Pro
 1               5                  10                  15

Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn
            20                  25                  30

Val Ile Leu Arg Lys His Arg Asn Met Val Val Arg Ala Cys Gly Cys
        35                  40                  45

His
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bos taurus

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Bovine genomic
(B) CLONE: Lambda 9800-10

(viii) POSITION IN GENOME:
(C) UNITS: bp

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 51..161

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 1..50

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 162..172

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 51..161

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
GGGGTGGGAG GGCACGTGGA TGGGACTCAC CTTCTCCCAC TACCCCCCAG GAC TGG          56
                                                          Asp Trp
                                                            1

GTC ATC GCC CCC CAA GGC TAC TCA GCC TAT TAC TGT GAA GGG GAG TGC         104
Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys
          5                  10                  15

TCC TTC CCG CTG GAC TCC TGC ATG AAC GCC ACC AAC CAC GCC ATC CTG         152
Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu
     20                  25                  30

CAG TCC CTG GTCAGTACCT C                                               172
Gln Ser Leu
 35
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly
  1               5                  10                  15

Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala
             20                  25                  30

Ile Leu Gln Ser Leu
         35
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bos taurus

vii) IMMEDIATE SOURCE:
(A) LIBRARY: Bovine genous
(B) CLONE: Lambda 9800-10

iii) POSITION IN GENOME:
(C) UNITS: bp

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 20..99

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 1..19

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 100..119

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 22..99

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
TGCGTG TCCCCGCAGA C GAC GTC CAC GGC TCC CAC GGC CGG CAG GTG        51
                    Asp Val His Gly Ser His Gly Arg Gln Val
                     1               5                  10

CGT CGG CAC GAG CTG TAC GTG AGC TTC CAG GAC CTG GGC TGG CTG        99
Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu
            15                  20                  25

GTTCCG ACTCTCCTTT                                                 119
```

INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His Glu Leu

Val Ser Phe Gln Asp Leu Gly Trp Leu
20 25

INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bos taurus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His Glu Leu
1 5 10 15

Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro
20 25 30

Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu
35 40 45

Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val
50 55 60

His Leu Leu Lys Pro His Ala Val Pro Lys Ala Cys Cys Ala Pro Thr
5 70 75 80

Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val
85 90 95

Ile Leu Arg Lys His Arg Asn Met Val Val Arg Ala Cys Gly Cys His
100 105 110

Supplemental Microorganism Sheet

International Application No: PCT/ US91 / 03388

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page _____, line _____ of the description [1]

A. IDENTIFICATION OF DEPOSIT [2]

Further deposits are identified on an additional sheet ☐ [3]

Name of depositary institution [4]

American Type Culture Collection

Address of depositary institution (including postal code and country) [4]

12301 Parklawn Drive
Rockville, Maryland 20852 USA

Name of Deposit	ATCC No.	Referred to on page/line	Date of Deposit
Lambda FIX/H8-12-1	75010	31/29	15 May 1991
Lambda EMBL/9800-10	75011	26/31	15 May 1991

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [5] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [6] (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later [9] (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau [10]

was

(Authorized Officer)

Form PCT/RO/134 (January 1981)

What is claimed is:

1. A purified BMP-8 protein comprising at least one of the following sequences:

a) Arg-His-Glu-Leu-Tyr-Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr (SEQ ID NO: 1);

b) Leu-Ser-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg (SEQ ID NO: 2);

c) Ala-Cys-Cys-Ala-Pro-Thr-Lys (SEQ ID NO: 3);

d) Thr-Asn-Glu-Leu-Pro-Pro-Pro-Asn-Lys-Leu-Pro-Gly-Ile-Phe-Asp-Asp-Val-His-Gly-Ser-His-Gly-Arg (SEQ ID NO: 4); and

e) sequences homologous to the sequences of a) through d) which encode the homologues of proteins characterized by the sequences of a) through d).

2. The protein of claim 1 further characterized by the ability to induce the formation of cartilage and/or bone.

3. A DNA sequence encoding a BMP-8 protein said DNA sequence comprising at least one of the following sequences

a)
GTG CAC CTG CTG AAG CCG CAC GCG GTC CCC AAG GCG TGC TGC GCG CCC ACC AAG CTG AGC GCC ACT TCC GTG CTC TAC TAC GAC AGC AGC AAC AAC GTC ATC CTG CGC AAG CAC CGC AAC ATG GTG GTC CGC GCC TGC GGC TGC CAC (SEQ ID NO: 7);

b)

GAC TGG GTC ATC GCC CCC CAA GGC TAC TCA GCC TAT TAC TGT GAA GGG GAG TGC TCC TTC CCG CTG GAC TCC TGC ATG AAC GCC ACC AAC CAC GCC ATC CTG CAG TCC CTG (SEQ ID NO: 9); and

c)

GAC GTC CAC GGC TCC CAC GGC CGG CAG GTG
TGC CGTCGG CAC GAG CTG AGC TTC CAG GAC CTG GGC TGG CTG (SEQ ID NO: 11).

4. A purified protein characterized by the amino acid sequence encoded by the DNA of ATCC #75010.

5. The DNA sequence of ATCC #75010 encoding BMP-8.

6. A purified protein produced by the steps of:

(a) culturing a cell transformed with a vector having a DNA sequence of claim 3 said DNA sequence in operative association with an expression control sequence therefor; and

(b) recovering, isolating and purifying from said culture medium a protein characterized by the ability to induce cartilage and/or bone formation.

7. A purified protein produced by the steps of:

(a) culturing a cell transformed with a vector containing the DNA sequence of ATCC #75010 encoding BMP-8 said sequence

in operative association with an expression control sequence therefor; and

(b) recovering, isolating and purifying from said culture medium a BMP-8 protein characterized by the ability to induce cartilage and/or bone formation.

8. A host cell transformed with a DNA of claim 3.

9. A host cell transformed with the DNA of claim 5.

10. A method for producing a BMP-8 protein said method comprising the steps of:

(a) culturing a cell transformed with a vector having a DNA sequence of claim 3 said DNA sequence in operative association with an expression control sequence therefor; and

(b) recovering, isolating and purifying from said culture medium a protein characterized by the ability to induce cartilage and/or bone formation.

11. A method for producing a purified BMP-8 protein said method comprising the steps of

(a) culturing a cell transformed with a vector having a DNA sequence of claim 5 in operative association with an expression control sequence therefor; and

(b) recovering, isolating and purifying from said culture medium a protein characterized by the ability to induce

cartilage and/or bone formation.

12. A pharmaceutical composition comprising an effective amount of a BMP-8 protein in admixture with a pharmaceutically acceptable vehicle.

13. A pharmaceutical formulation for bone and/or cartilage formation comprising an effective amount of a BMP-8 protein in a pharmaceutically acceptable vehicle.

14. A composition of claim 13 further comprising a matrix for supporting said composition and providing a surface for bone and/or cartilage formation.

15. The composition of claim 14 wherein said matrix comprises a material selected from the group consisting of hydroxyapatite, collagen, polylactic acid and tricalcium phosphate.

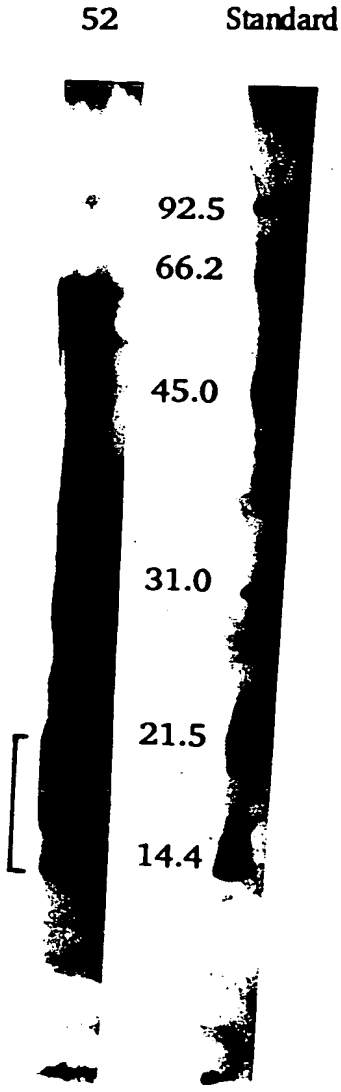
16. A pharmaceutical composition for wound healing and tissue repair said composition comprising an effective amount of a BMP-8 protein in a pharmaceutically acceptable vehicle.

ABSTRACT

Purified cartilage and/or bone inductive proteins and processes for producing them are disclosed. The proteins may be used in the treatment of bone and/or cartilage defects and in wound healing and related tissue repair.

PCT/US 91/05500
RO/US 22 JUL 1991

Figure 1



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